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TITLE: A Study of Transrectal Tumor Oxygen Measurements in
Patients with Clinically Localized Prostate Cancer

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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) The primary aim of this clinical study is to determine the relationship between pre-treatment prostate cancer oxygen levels and long-term disease control following treatment with radiotherapy, and the independent prognostic effect of oxygen measurements relative to established prognostic factors. In addition, the study will determine the relationship between pre-treatment tumor oxygen levels and mutations of the p53 tumor suppressor gene, and the impact of this interaction on patient outcome. A total of 40 patients were accrued to the study in year 1. This is below the anticipated accrual of 65 patients per year. Measures have been implemented to increase accrual in year 2, and the ultimate goal of determining the independent prognostic significance of tumor oxygenation in patients with prostate cancer will be achieved. The micro-regional distribution of oxygen in prostate cancer biopsies will be studied using one of the intrinsic markers of oxygenation rather than EF5 as described in the initial proposal. The molecular studies of p53 and apoptosis are proceeding as outlined in the proposal.				
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Introduction

Hypoxia is known to impair the effectiveness of both surgery and radiotherapy at curing a variety of solid human tumors. This may result from an hypoxia-induced increase in genetic instability leading to altered expression of genes that are important in tumor growth and progression. These genetic changes are manifested clinically as more aggressive tumor behavior. The primary aim of this clinical study is to determine the relationship between pre-treatment prostate cancer oxygen levels and long-term disease control following treatment with radiotherapy, and the independent prognostic effect of oxygen measurements relative to established prognostic factors. In addition, the study will determine the relationship between pre-treatment tumor oxygen levels and mutations of the p53 tumor suppressor gene, and the impact of this interaction on patient outcome.

Body

Progress in the first year of the research award has focused on Task 1 of the Statement of Work:

Task 1. Accrual of patients (years 1-3)

Patients will be accrued to this study at a uniform rate of 65 per year (52 eligible patients per year allowing for 20% attrition), over the three years from January 2001 to December 2003. Clinical and surgicopathologic prognostic information will be collected prospectively at the time each patient enters the study. Eppendorf oxygen measurements will be made. A biopsy will be obtained immediately after the oxygen measurements and evaluated for mutations of the p53 tumor suppressor gene and apoptosis. The biopsies will be processed in batches during the accrual period. A portion of each biopsy will be stored for future study of other hypoxia-related genes.

Patients will be accrued to the EF-5 component of the study in the second and third years once phase I testing of this agent is complete in Canada.

Accrual to this clinical study began in August 2001 after the protocol was approved by the Human Subjects Research Review Board of the U.S. Army Medical Research and Materiel Command, and our local institutional Research Ethics Board. A total of 40 patients were accrued in the first year. All patients had trans-rectal needle-electrode measurements of prostate cancer oxygenation and prostate biopsies as described in the protocol, and received high-dose conformal radiotherapy.

The expected accrual to this study was at the rate of 65 patients per year over 3 years, to achieve a total of 156 eligible patients after allowing for attrition. Accrual to date has been slower than anticipated, at 65% of predicted. This is attributable to several factors that could not have been anticipated in advance, including staff absences, measurement equipment malfunction and a short-term 20% reduction in the number of patients receiving radiotherapy for prostate cancer at this institution to facilitate replacement of aging radiation treatment equipment. We have introduced a number of procedures in an attempt to increase accrual to the expected level of 65 per year:

1. Potentially eligible patients will be identified by a research assistant in advance of their first clinic appointment, as a reminder to the attending radiation oncologist about the study. The radiation oncologist will introduce the study to the patient as part of the general discussion about radiotherapy.
2. The research assistant will spend whatever time is necessary with the patient and his family explaining the study and the consent form, in an attempt to allay any concerns about the study.
3. A clinical research fellow has been designated for this project and will also be responsible for identifying and accruing eligible patients.
4. All of the oxygen measurements have to this point been done by a single ultrasonographer, in order to assure consistency of the technique from patient to patient. However, patients were lost to the study when this individual was absent from the hospital to attend academic meetings or for vacation. A second experienced ultrasonographer has now been recruited to perform the studies at these times.
5. The oxygen measurement equipment has been over-hauled and is now functioning reliably.
6. The number of potentially eligible patients with prostate cancer who receive conformal radiotherapy at this institution is expected to increase over the next year from 250 to 300 as a result of improvements in equipment and process efficiencies. Therefore, the patient base is more than adequate to achieve the target accrual.

A minor component of the project involves the use of the hypoxia marker EF5. As outlined in Section 4.6 of the protocol, this was to be administered to 30 patients in years 2 and 3 to evaluate the microscopic distribution of oxygen in prostate cancer, and differences in gene expression between oxic and hypoxic regions. EF5 was in Phase I clinical testing in the United States and Canada at the time that the protocol was developed. The Phase I study of EF5 was independent of our prostate cancer study, and was funded from other sources. The EF5 component of our prostate study was not submitted to either the U.S. Army Human Subjects Review Board or our local institutional

Research Ethics Board pending completion of Phase 1 testing to establish the safety of this agent in humans. No patients have been accrued to the EF5 component of the study.

The Phase I study of EF5 was abandoned in Canada approximately 1 year ago due to lack of accrual. EF5 needs to be administered intravenously to patients 24 to 48 hours before biopsy of the tumor. This proved to be impractical, and many patients were unwilling to accept an intravenous injection of an experimental drug. Therefore the EF5 component of our prostate study will not proceed as initially planned. Nevertheless we will be able to achieve the goals initially set out in the protocol. Over the past 2 to 3 years there has been significant development in the area of intrinsic hypoxia markers. These are normal proteins which are known to be up-regulated in the setting of hypoxia, and can be used in the same manner as EF5 to evaluate micro-regional aspects of tumor oxygenation. The intrinsic markers have several advantages, in that administration of an external agent prior to biopsy is not required and the immunohistochemical analysis can be done on previously-obtained paraffin embedded tissue. We propose to use intrinsic markers of hypoxia instead of EF5 to accomplish the goals of this project. Candidate markers, including carbonic anhydrase IX (CA-IX), glucose transporter (GLUT-1) and hypoxia inducible factor-1 α (HIF-1 α) [1-5] are presently being studied in prostate cancer xenografts, and the most promising of these markers will then be applied to the patient biopsy specimens. The analysis technique for the intrinsic markers will not differ significantly from that originally proposed for EF5, and we therefore do not anticipate any change to the budget.

The studies relating to the p53 status of the human tumors (DNA sequencing) and immunohistochemical endpoints of p53 related pathways (section 4.5 of the protocol) are entering their post-development stage. The first year of the project was devoted to creating selective primers for the DNA sequencing method, so that all eleven exons of the p53 gene could be sequenced. We have now laser-capture micro-dissected material from both formalin and frozen histologic sections using biopsies taken from patients prior to radiotherapy, and have sequenced all exons of the p53 gene by PCR-based DNA sequencing technologies. Furthermore, initial studies have supported the use of p53 and apoptosis-related (bax, bcl-2, survivin and TUNEL assays) immunohistochemical markers in relation to the track lengths used for the needle-electrode oxygen measurements. Sufficient RNA has been amplified from laser-capture micro-dissected biopsies to form the basis for future hypothesis-generating experiments utilizing DNA microarrays, and for in situ hybridization measurements of p21 WAF expression.

To date, the p53 gene sequencing and related immunohistochemical studies have been completed for 10 patients. We have recently acquire a new laser capture microdissector in our institution, which will allow more rapid throughput of human tissues (decreasing dissection time from 3 hours to 60 seconds per patient). We will be analyzing 10 patients per month over the next 12-18 months to drive correlative studies of hypoxia and gene expression in the patient cohort.

Task 2. Follow-up (years 4-7)

Patients will be followed for a duration of 3.5 years after completion of accrual in order to realize the required 46 PSA relapses. Patients will be assessed clinically and have PSA measured at regular intervals as part of their routine medical care. The database will be updated on an ongoing basis to reflect current disease and patient status.

Patients who were accrued to the study in year 1 are being followed after the oxygen measurements and radiotherapy as outlined in section 4.8 of the protocol.

Task 3. Analysis (years 4 and 7)

The comparison of tumor oxygenation to other clinical and surgico-pathologic prognostic factors will be done after completion of accrual (early in year 4). The analysis of the influence of tumor hypoxia on outcome will be done after patients have been followed for an additional 3.5 years (mid year 7).

No analysis of the data has been undertaken.

Key Research Accomplishments

Ongoing patient accrual

Reportable Outcomes

None to date

Conclusions

This study of oxygenation in human prostate cancer is accruing patients, although at a somewhat slower rate than originally anticipated. Measures have been implemented to increase accrual in year 2, and the ultimate goal of determining the independent prognostic significance of tumor oxygenation in patients with prostate cancer will be achieved. The microregional distribution of oxygen in prostate cancer biopsies will be studied using one of the intrinsic markers of oxygenation rather than EF5 as described in the initial proposal. The molecular studies of p53 and apoptosis are proceeding as outlined in the proposal.

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